Phase I/II clinical trial of local GM-CSF application in patients with cervical HPV-associated low-grade squamous intraepithelial lesions (SIL).

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Introduction

Quantitative and functional alterations of professional antigen-presenting cells (APC) in SIL suggest that these lesions may have a diminished capacity to capture viral antigens. Moreover, GM-CSF (whose production is decreased in HPV-transformed keratinocytes) is an essential factor for the migration of APC in cervical (pre)neoplastic lesions formed *in vitro* and transplanted in vivo on mouse. In this study we performed a phase I/II clinical trial in order to determine whether a local application of GM-CSF on cervical low-grade squamous intraepithelial lesions (LSIL) might increase the recruitment of APC into the epithelium and indirectly the viral antigen presentation to the immune system.

Table 1: List of women enrolled in clinical trials

G1-G10 patients receiving GM-CSF; P1-P5 placebo patient

N°	Age	Cervix	HPV type	Viral load before treatment
G1	41	LSIL	59/70	21
G2	34	LSIL	35	955
G3	33	LSIL	18	11
G4	45	LSIL	16/31/53	1183
G5	41	LSIL	16/56/66	74
G6	36	LSIL	56/59/66	37
G7	29	LSIL	43/44-55/52	30
G8	47	LSIL	42/44-55/52/53/70	133
G9	35	LSIL	53/56	4
G10	31	LSIL	16/52	410
P1	27	LSIL	31/44-55/53	37
P2	30	LSIL	39	0
P3	37	LSIL	68	7
P4	21	LSIL	56/66	40
P5	29	LSIL	66	6

Materials and methods

Clinical protocol: 15 patients with LSIL (10 GM-CSF and 5 placebo) were enrolled in this study **(table 1).** At visit A (week -35 to -5) and B (week -21 to -1) a colposcopy and sampling for viral detection and DNA load determination were performed. The confirmation of LSIL was performed on biopsies obtained either at visit A or B. GM-CSF (4 applications, 800.000 IU/ml) or placebo gel was applied at visit C (Week 0). Follow up was performed at visit D (week 2), visit E (week 6 to 15) and F (week 23 to 42).

APC and CTL infiltration was quantified by immunostaining with anti-CD1a and CD8 mAb respectively. **Cellular immune response** was evaluated by using an IFN-γ intracellular staining on PBMC stimulated *in vitro* with the E7 HPV16 protein and L1 HPV16 Virus-like particles (VLP).

The **HPV genotyping** was determined on cervical brush specimens using PapilloCheck Test Kit (Greiner Bio One). Hybrid capture Digene was performed to **semi-quantify the viral DNA** in cervical brush specimens. Since the test was conducted in a double-blind way, we observed at the end of test that placebo patients (29 ± 6 years) was significantly younger than the patients receiving the GM-CSF (37 ± 6 years, p< 0.05) **(Table 1).**

Results

GM-CSF applications were well tolerated in all patients. No difference in the cytological /histological and viral parameters assessed at 2 and 6 months **(Fig. 1)** after the last application was observed between the GM-CSF and the placebo group. An increased number of CD1a+ APC was observed in 8/10 patients treated by GM-CSF compared to 1/5 patient in the placebo group **(Fig. 2 A-C)**. We also observed a higher infiltration of CTL (CD8+) in GM-CSF treated-patients **(Fig. 2 D)**. In the patient cohort, before the treatment only 3 patients were HPV16+ in the GM-CSF-treated group and none in the placebo group. All these 3 patients exhibited an immune response against HPV16 after GM-CSF application as showed by NK and/or T cells producing IFN-γ after stimulation with E7 HPV16 or VLP HPV16. **(Fig. 3)**. We also tested the anti-HPV16 response in PBMC from patients negative for HPV16; a trend of a higher percentage of lymphocytes expressing IFNγ after stimulation with HPV16 L1-VLP was observed in GM-CSF group compared to placebo patients **(Fig. 4)**.

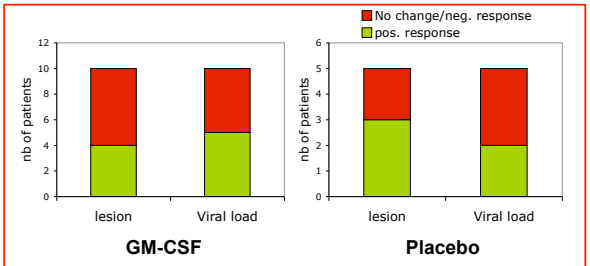

Figure 1: Clinical response

The evaluation of the lesion grade was assessed by cytological examination of Papanicolaou stained cervical smears and histologicaly on cervical biopsies. Viral load was quantified by Hybrid capture.

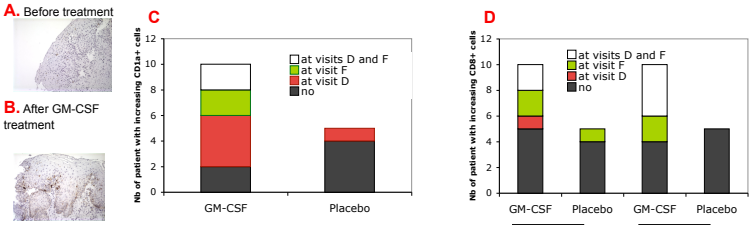

Figure 2. Infiltration of APC (CD1a+) and CTL (CD8+) in cervical LSIL.

Representative pictures of immunostaining of CD1a+ cells before **(A)** and after GM-CSF treatment **(B)**. Number of patients with increased infiltration of CD1a+ cells at week 2 and/or week 23-42 after application of GM-CSF or placebo gel **(C)**. Number of patients with increased infiltration of CD8+ cells in the epithelium or in the stroma at week 2 and/or week 23-42 after application of GM-CSF or placebo gel **(D)**.

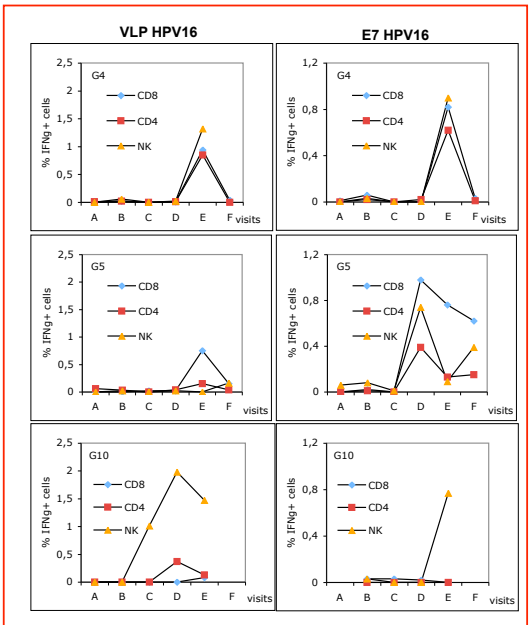

Figure 3. IFNγ production in response to HPV16 E7 or HPV16 L1-VLP in HPV16 positive patients.

PBMC were stimulated overnight with HPV16 E7 or L1-VLP and the percentage of IFNγ+ cells was determined in CD4+, CD8+ or CD56+CD16+CD3- (NK cells) gated cell populations.

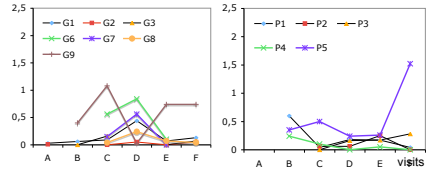

Figure 4. IFNγ production in response to HPV16 L1-VLP in HPV16 negative patients.

PBMC were stimulated overnight with HPV16 VLP L1 and the percentage of IFNγ+ cells was determined in lymphocyte gated cell populations.

These encouraging results obtained from a limited number of subjects justify further analysis of the therapeutic effect of GM-CSF in cervical preneoplastic lesions.

Acknowledgements

RÉGION WALLONNE

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